

CHEMICAL NATURE OF ANTHRACNOSE RESISTANCE IN CUCURBITS

by

ANNA CECILIA MATHILDA BREDENBERG

B. Sc. (Ag.), University of Helsinki, Finland, 1957

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1961

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INTRODUCTION

The fungus Colletotrichum lagenarium (Pass.) Ell. and Halst. is a widely recognized pathogen which causes anthracnose in cucurbits. This pathogen may attack any above-ground part of host plants of the species cucumber, Cucumis sativus L.; watermelon, Citrillus vulgaris Schrad; muskmelon, Cucumis melo L.; Cucurbita moschata Duch.; C. maxima Duch., and C. pepo L.

Anthracnose is common in Europe and America, generally in areas with frequent rainfall and high humidity, since the causal organism depends upon water for sporulation. Brown to black lesions appear on leaves. On stems and petioles the lesions are elongated, slightly sunken, watersoaked and yellowish, but later becoming dry and dark. Girdling of stems and petioles often occurs, especially in young seedlings (5). The heaviest economic losses are observed to occur on fruits of watermelon after harvest, in storage and transit (32). Lesions may not appear on fruits until they approach maturity when sunken, watersoaked lesions expand to considerable size with black acervuli in the center. The acervuli are fruiting bodies of the fungus and produce pinkish spore masses in moist weather (32).

The problem of the nature of host resistance has been approached from three different standpoints: 1) morphological plant characters, 2) host metabolites toxic to the pathogenic agent, and 3) lack of essential nutrients in the host required by the pathogenic agent. In recent studies the two latter fields of research have gained more attention and the basis of the nature of host resistance is thought to be found there while morphological characters are suggested to be only of secondary importance. However, very little is known about the chemical nature of host resistance. Genes that govern resistance have been identified (19), but there is little information about the

function of these genes in the biochemical reaction of the host plants.

The purpose of this study was to observe reactions of races and biochemical mutants of the fungus Colletotrichum lagenarium when cultured 1) on the surface of intact seedlings, 2) in epidermal tissue homogenate and 3) in whole or subepidermal tissue homogenates.

REVIEW OF LITERATURE

The disease was first described by Passerini in 1867 on fruits of gourd, Lagenaria vulgaris at Padua, Italy. In England in 1871 the fungus was observed to attack cucumbers, and somewhat later watermelon and muskmelon (5). The occurrence and importance of anthracnose in the United States was first reported by Scribner (29) in 1888.

The early workers recorded the fungus as a species of the genus Gloeosporium. This conception was corrected by Gardner (16) in 1918, who proposed the present name Colletotrichum lagenarium. This name refers to the imperfect stage of the fungus. The perfect stage was not recognized until 1952. The fungus was found in Japan to be an ascomycete and was given the name Glomerella lagenaria Watanabe et Tamura (33). G. lagenaria has not been reported in the United States.

Breeding of resistant varieties has gained in importance, since cultural and chemical control measures seldom give satisfactory results. The study of inheritance of the host resistance, and the variability of the pathogenicity of the fungus have been the main characteristics to be observed.

Layton (25) was the first to report that a single pair of dominant genes may be responsible for anthracnose resistance in the watermelon. This report was later confirmed by Hall, et al. (19), who stated that resistance was

conditioned by a single dominant gene so far as the Kansas strain of C. lagenarium was concerned. In experiments carried out by Kalia (21) the watermelon varieties Congo, Fairfax, and Charleston Gray appeared resistant to this strain. However the resistance of Charleston Gray was observed to be of somewhat lower degree than the resistance of the two other varieties mentioned. Quite recently Hall, et al. (18) reported variability within all resistant and susceptible varieties of watermelon.

Also, cucumber varieties show differences in resistance to the same organism. Resistance of a monogenic character was reported by Barnes and Epps (2). Busch and Walker (4) have mentioned multigenic resistance in the cucumber line, PI 163217, and a case of either incomplete dominance of a resistant gene or the presence of modifying genes in PI 163213.

The fact that resistant varieties suddenly may become susceptible has turned the attention to the occurrence of natural pathogenic races of C. lagenarium. In 1958, Goode (17) reported information about three different races which culturally and morphologically were similar, and were distinguished only on the basis of their physiological specialization. He observed differential host reactions in Butternut squash, Charleston Gray watermelon, and PI 163213 cucumber. These three varieties appeared helpful in the identification of the races and have since then been used in research concerning pathogenic variability and resistance. However Dutta, et al. (9) found a difference in the reaction of the same races on the same host varieties. However they reported that the varieties mentioned by Goode still permitted the races to be identified. A fourth race of C. lagenarium was described for the first time (9).

Response of the pathogen to resistant and susceptible host tissue has been

studied by Akai, et al. (1). Mature leaves of both resistant and susceptible varieties of squash and cucumber were invaded by C. lagenarium in an equal manner. The difference in susceptibility was correlated to mycelial development and penetration after invasion. After establishment, however, the rate of lesion and mycelial development appeared as good in the resistant as in susceptible plants. These observations indicate that the factor of resistance must be sometime between the penetration of host tissue and the establishment of the pathogen. Yasumori (35) found that wounding of cucumber plants in no case removed resistance.

The necessity of a comprehensive study of the mechanism of penetration in the fungus Colletotrichum lagenarium became evident. Busch and Walker (4) confirmed that penetration occurs equally well in resistant and susceptible plants. The penetration peg invaded the epidermal cell and the mycelium invaded the mesophyll cells intracellularly. However, the progress of the hyphae was much slower in the resistant tissue. The morphological reactions in resistant plants appeared as a thickening of the cell walls in advance of the hyphae, and showed up as red-staining deposits in the intercellular spaces. The cell contents of resistant tissue reacted more slowly than those of susceptible tissue in advance of the fungus penetration. It was assumed that "the true basis of resistance may well be biochemical, with morphological response a purely secondary reaction" (4).

The presence or production of certain materials in the host, inhibitory to pathogens has been proved in several cases. Water soluble phenols, protocatechuic acid and catechol diffusible to the dry outer scales of onion bulbs account for the resistance in onions to infection by the smudge fungus Colletotrichum circinans (Berk.) Vogl. and the neck rot fungus Botrytis

allii Munn. (30). According to Menon and Schachinger (27), the production of phenols in tomato plants is proportional to the degree of resistance to fusarium wilt. The increased polyphenoloxidase activity is to be considered as a host defence reaction. Further, the resistance of carrot root tissue to attack by the fungus Ceratocystis (Ceratostomella) fimbriata (Ell. and Halst.) Hunt is related to the production of a phenolic ester by the roots after inoculation (7).

Johnson and Schaal (20) correlated the presence of chlorogenic acid in the peel of white potato tubers with resistance to potato scab, caused by Streptomyces scabies (Thaxt.) Waksman and Henrice. Chlorogenic acid has also been suggested as a possible mechanism for the resistance of certain sweet-potato varieties to attack by Ceratocystis fimbriata (31). An amino acid addition product of chlorogenic acid, present in extracts of white potato peel, has appeared highly toxic to Race I of Helminthosporium carbonum Ullstrup. Breakdown of the product yields: chlorogenic acid, caffeic acid, and isoleucine, methionine, phenylalanine, tryptophane, tyrosine, valine, and two more nonidentified amino acids. Coupled with the breakdown a marked decrease in the inhibitory activity can be noted (6). According to Kuc, et al. (23) the D and DL isomers of phenylalanine alone, markedly increased the resistance of seven apple varieties to apple scab caused by the fungus Venturia inaequalis (Cke.) Wint. Lewis (26) showed that the amino acids cystine and alanine are fairly constant inhibitors of growth of Alternaria solani (Ell. and G. Martin) Sor., which causes the early blight of tomato.

Lakshminarayan (24) related the fusarium wilt resistance in certain cotton varieties to the presence of an amino acid, cystine. Cystine was absent in susceptible varieties. But two host varieties with different degrees of

resistance do not necessarily differ in their chemical composition previous to infection. Rohringer, et al. (28) working with fusarium wilt showed that resistant and susceptible varieties of healthy tomato plants contained the same amounts of free amino acids, sugars, some acidic components, and phenols. Following infection, a great number of components changed in concentration in plants of the susceptible variety; little or no change was observed in resistant plants. Attention was directed to an acidic component of the host tissue, an organic phosphate which was affected in a way that suggested its relation to resistance. In this connection it may be emphasized that only metabolites of the host play a role in selectivity of forms for specific host plants and in resistance of varieties within species. Metabolites produced by the pathogen, enzymes or toxic acids are disease inducing agents which affect symptom expression. Resistance cannot be explained on a basis of inactivation of these disease producing metabolites (18, 28, 34).

Since 1956 Garber's "nutrition-inhibition hypothesis of pathogenecity" has been commonly accepted. According to this hypothesis a pathogen is virulent only when an adequate nutrition - ineffective inhibition environment is prevailing at the site of inoculation or of localization (11). The chemical nature of disease resistance appears to be a very complicated subject. Much research with plant and animal pathogens as well has been done. Biochemical mutants obtained by means of ultra violet irradiation (3, 15) have become valuable tools in studying the role of nutrition in different pathogens. Working with the bacteria Erwinia ardoideae (Townsend) Holland, a soft rot organism, common to several vegetable crops Garber (10) studied the availability of the nutrilites using different inoculation technics. The fact that the required nutrilites may be present in the host tissue but still not available

to the parasite, and no toxic metabolites of the host are detected suggests the presence of unknown inhibitors.

Garber and Goldman (13) carried out further study with biochemical mutants of Erwinia aroidae on grape tissue cultures and obtained similar results. Garber and Shaeffer (14) correlated resistance to histidine mutants of the same bacteria with a low histidine concentration in the fleshy storage organs of turnip varieties. However, one year later the validity of this report was canceled. The difference in histidine content in resistant and susceptible varieties was assumed to be the result of non-genetic (environmental) factors (12). Some of the histidine requiring mutants showed avirulence for a few varieties with adequate concentrations of histidine for proliferation and metabolism. It may be assumed that the resistance would be associated with an inhibition of the uptake of histidine (12). Kline, et al. (22) produced clear evidence concerning the solidity of the nutrition hypothesis. They were able to restore the pathogenicity of six avirulent mutants of Venturia inaequalis by an exogenous supply of required nutrients. A study with natural races and induced biochemical mutants of Colletotrichum lagenarium has recently been accomplished by Dutta (8). He restored the pathogenicity of histidine, serine, leucine, isoleucine and proline deficient mutants, but those requiring pyrimidines could not be restored by Dutta.

MATERIALS AND METHODS

This study, which was conducted between February and July 1960, deals with the chemical nature of anthracnose resistance in cucurbits. The purpose of this work was to observe the growth variations of races and mutants of Colletotrichum lagenarium, when cultured on 1) the surface of intact seedlings,

2) epidermal tissue homogenates, and 3) whole or sub-epidermal tissue homogenates. Attention was paid to the nutritional requirements of the fungus as they may or may not be supplied by the host. A search was made for the site of the resistance in the cucurbit host tissue. Five experiments were completed in the Horticultural greenhouse and research laboratory of the Department of Botany and Plant Pathology, Kansas State University.

Plant Culture

The following differential host varieties were used: watermelon, Charleston Gray (resistant) and Black Diamond (susceptible); squash, Butter-nut (resistant); and the cucumber line PI 163213 (resistant). However PI 163213 was discontinued in experiments four and five since the seed supply was exhausted.

Plants for all experiments were grown in two inch clay pots filled with a soil mixture containing two parts field soil, one part sand and one part peatmoss. Pots containing the soil mixture were steam sterilized. Seeds were planted in the pots and placed in flats which were lined with plastic so as to maintain a high soil moisture level. The plants were watered once daily by running water into the plastic lined flats so it could be absorbed through the bottom of the pots. Two seedlings were grown per pot and a total number of 600 seedlings were planted for each experiment except for experiment three where 800 seedlings were planted. Two to four non-supplemented plants and two to three supplemented plants were used for each mutant and three to four for each race. Seedlings which were used in the greenhouse tests were similar to those shown in Fig. 1.

EXPLANATION OF PLATE I

Fig. 1. Seedlings typical of those which were used for the greenhouse experiments.

Fig. 2. Inoculated seedlings in the moist chamber.

PLATE I



FIG. 1



FIG. 2

Pathogen Culture and Preparation of Inoculum

Four natural races with different degrees of virulence to the various host varieties were used. One was isolated in Kansas (KS II), the three others in North Carolina (R I, R II, R III). Race II is virulent to all of the known resistant watermelon varieties. The artificial biochemical mutants were developed by Dutta (8) from Race II of *C. lagenarium*. The nutritional requirements of the mutants that were used in this study were as follows:

<u>Mutant number</u>	<u>Requirements</u>
	<u>Pyrimidines</u>
44	Uracil or Cytosine
52	Uracil or Cytosine
	<u>Amino Acids</u>
22	Proline
42	Leucine
4	Isoleucine
24	Histidine
29	Serine
48	Alanine
49	Valine
	<u>Vitamins</u>
2	Inositol

The fungus was cultured in test tubes on standard potato dextrose agar (PDA). Fresh cultures were provided for every experiment two to three weeks before inoculation to obtain a maximum rate of sporulation.

Spore suspensions were prepared previous to inoculation by pouring five cc. of sterile distilled water into each test tube and shaking the tube until the spores were in suspension.

Dilution of Nutrilites

Table 1. Nutrilite dilutions for mutant supplements.

Mutant number	:	Supplement	:	Molar concentration
44		Pyrimidines		1×10^{-3}
52		Pyrimidines		1×10^{-3}
22		L-Proline		1×10^{-3}
42		L-Leucine		8×10^{-4}
4		L-Isoleucine		8×10^{-4}
24		L-Histidine HCL		7×10^{-4}
29		DL-Serine		2×10^{-3}
48		Alanine		3×10^{-3}
49		Valine		1×10^{-3}
2		Inositol		2×10^{-3}

Forty cu. cm. of solution was made up for each nutritive. The solutions were then filtered through "Seitz" filters and poured into sterile dropper bottles. The droppers were previously calibrated to 0.5 cc. in order to supply consistent amounts of the nutritives.

Tissue Homogenates

The tissue homogenates were prepared by grinding epidermal, sub-epidermal, and whole tissues separately for five minutes in a Waring blender which contained 120 to 200 cc. of distilled water, depending upon quantity of fresh plant material used for the respective experiments. The homogenates were filtered twice through double filter paper, first using coarse and last fine

filter paper. In the final filtering, sterile "Seitz" filters connected to sterile separatory flasks were used for the purpose of sterilizing the tissue homogenates. All equipment, including the "Seitz" filters, test tubes with cotton stoppers, a calibrated B-D Multifit control syringe with an attached tube, etc., was sterilized before each experiment and between the preparation of the homogenates of different hosts and different tissues.

After filtering was completed two cc. of each homogenate was transferred with the Multifit syringe to each of two to four test tubes per mutant and race which were kept non-supplemented. One and one-half cc. of each homogenate was used in each of two to three tubes per mutant. To these 0.5 cc. of the required nutrilites were supplemented after inoculation, thus giving an equal amount of liquid in all tubes.

Inoculation Technics and Incubation

Seedlings. Two different methods of inoculation were used for seedlings. First, the spore suspension was applied directly from the test tube cultures to cheese cloth squares which were placed on the leaves (Plate I, Fig. 2 and Plate II, Fig. 2). The second method was the "dunking" technic which was used in the first and the last experiment. This spore suspension prepared as previously described, was then poured into a container to which a few drops of Tween 80 was added as an emulsifier. Seedlings in the pots were then dipped into the suspension. Cheese cloth squares were then placed on the leaves and in both methods the nutrilitie supplements were placed on the cheese cloth with a dropper.

Following inoculation the seedlings were placed in a moist chamber in which the temperature was maintained at about 85 degrees F. and the relative humidity

EXPLANATION OF PLATE II

- Fig. 1. Fungus growth ratings in tissue homogenates are from left to right 0, trace, +, ++, and +++ respectively.
- Fig. 2. Symptoms on seedling leaves covered with cheese cloth squares.

PLATE II



Fig. 1



Fig. 2

EXPLANATION OF PLATE III

Seedlings of Black Diamond (Fig. 1) and Charleston Gray
(Fig. 2) showing symptoms of infection by races I, II,
III and KS II from left to right.

PLATE III



Fig. 1



Fig. 2

above 80 per cent.

Homogenates. Inoculation of the homogenates in the laboratory was first done in an inoculation chamber using a loop needle. Since this was slow, a new method using sterile pipettes was developed in which one to two drops of the spore suspension was dropped into each test tube. Sterile pipettes were provided for each mutant or race. Tubes were then incubated in the laboratory at room temperature.

Rating Scales

Seedlings. Seedling ratings were made about ten days after inoculation. Symptoms caused by the races were rated according to the following scale which is similar to the one reported by Kalia (21) and later by Dutta (8):

<u>Degree of infection</u>	<u>Scale</u>	<u>Description of symptoms</u>
Immune	0	No infection; no symptoms
Highly resistant	1	Slight infection; a few lesions on any above ground parts of plants.
Resistant	2	Medium infection; large lesions.
Moderately resistant	3	Comparatively severe infection but plants still alive.
Susceptible	4	Very severe infection; girdling around petioles and stems.
Highly susceptible	5	Rapid death of plants.

Symptoms caused by the mutants were rated by a different system since the lesions resulting from infection by the respective mutants were smaller. This scale may be outlined as follows:

<u>Degree of infection</u>	<u>Scale</u>	<u>Description of symptoms</u>
Immune	0	No symptoms; no fungus growth
Extremely resistant	tr	A very small lesion; trace of fungus growth.
Highly resistant	+	One lesion; some fungus growth.
Resistant	++	One large lesion or two to three small ones; evident fungus growth.
Moderately resistant	+++	Two to three large lesions or girdling of petioles; fungus growth expanded throughout the homogenate suspension.

Homogenates. Ratings of fungus growth in tissue homogenates were made according to the "0 to +++" scale as described above, (Plate II, Fig. 1).

Experiments

Experiment One. This experiment was started on February 12, 1960. It was a preliminary experiment for the purpose of refining experimental technics and greenhouse conditions. The only host variety used was Charleston Gray, and only the mutant strains of the pathogen. No results are included for this experiment.

Experiment Two. The laboratory test was started February 18, 1960. All four host varieties were included as well as mutants and races. When the plants reached the four to six true leaf stage they were used for the whole tissue homogenate. This was prepared by grinding leaves, stems, and roots of fifteen seedlings in 200 cc. of distilled water. The homogenate was transferred to two test tubes per non-supplemented mutant and race and to the same number of tubes per supplemented mutant. The homogenates were inoculated on March 19, and then incubated in the laboratory at room temperature for nine

days. Two ratings were made, one after five, and the second after nine days. Readings were similar but the mycelial growth appeared darker at the latter rating which made the variations easier to distinguish.

The greenhouse phase was initiated on February 25. All four host varieties were included with only the mutants of the fungus being evaluated. Seedlings in the two true leaf stage were inoculated on March 20. The inoculum and the required nutrilites were applied to the leaves on cheese cloth squares. Readings were made on April 11 according to the "0 to +++" rating scale.

Experiment Three. All four host varieties, the ten mutants and the four races of the fungus, were included in both sections of this experiment. Seedlings for the laboratory section were planted March 23. The epidermis was peeled off the stems and petioles for the epidermal tissue homogenate. Sub-epidermal tissues were used for a separate homogenate. Three gms. of fresh plant material was ground in 120 cc. of distilled water for each homogenate. Inoculation of the Black Diamond and the Butternut homogenates were made on April 15; and on April 23 for Charleston Gray and PI 163213. Sterile pipettes were used instead of the inoculation needle. The test tubes were then incubated at room temperature as in experiment two. Ratings were made eight days later according to the "0 to +++" scale.

Seedlings for the greenhouse section were planted on March 30. The squash and cucumber seed germinated earlier than those of the watermelon varieties, and reached the one to two true leaf stage about eight days earlier. Thus the two former varieties were inoculated on April 15, and the watermelon varieties on April 23. The inoculum and the respective supplements were applied to cheese cloth squares on the leaves. Plants were incubated in the moist chamber as previously described. Rating of all four host varieties

were made on May 3, the plants inoculated with mutants according to the "0 to +++" rating scale, and races according to the "0 to 5" scale.

Experiment Four. Seedlings were planted in the greenhouse on April 29. The host plants used were Black Diamond, Charleston Gray, Butternut and, in five cases out of fourteen, cucumber 163213. Seedling inoculations were made on May 14 with both mutants and races using the cheese cloth squares on leaves. Ratings were made on May 31, as in experiment three.

Experiment Five. Seedlings were planted for both greenhouse and laboratory tests on June 8. In the laboratory phase the host varieties Black Diamond, Charleston Gray and Butternut were used but the cucumber line 163213 was omitted due to inadequate seed supply. Only the epidermal tissue homogenate was prepared and using the same concentration as in experiment three. The sub-epidermal tissue homogenate was omitted, since the growth response of the fungus in experiment three was very similar to the growth response in the whole tissue homogenate of experiment two. The plants were inoculated on June 30 with mutants and races and incubated as in experiment three. Ratings were made July 7 according to the "0 to +++" scale.

Seedlings of the watermelon varieties Black Diamond and Charleston Gray were inoculated with mutants and races on June 20 by the "dunking" technic. Butternut squash was omitted since the seedlings were consistently immune in all previous experiments. Incubation and ratings which were made on July 1 were the same as in previous greenhouse experiments.

EXPERIMENTAL RESULTS

Seedlings

Results obtained from greenhouse experiments dealing with seedlings are reported in Table 2.

Table 2. Seedling infections obtained by mutants of Race II of *C. lagenarium* in experiments two, three, four, and five, Manhattan, Kansas, 1960.

:Charleston Gray: Black Diamond : Butternut :Cucumber 163213
Experiments:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl

Pyrimidine 44								
Exp. Two	0	0	0	0	0	0	0	0
Exp. Three	0	0	0	0	0	0	0	0
Exp. Four	0	0	0	0	0	0	-	-
Exp. Five	-	-	-	-	-	-	-	-
Average	0	0	0	0	0	0	0	0

Pyrimidine 52								
Exp. Two	0	0	0	0	0	0	0	0
Exp. Three	0	0	0	0	0	0	0	0
Exp. Four	tr	+	0	++	0	0	-	-
Exp. Five	0	+	0	0	-	-	-	-
Average	0	tr	0	tr	0	0	0	0

Proline 22								
Exp. Two	0	0	+	+	0	0	0	0
Exp. Three	0	0	0	0	0	0	0	0
Exp. Four	0	+	+	++	0	0	-	-
Exp. Five	0	0	0	0	-	-	-	-
Average	0	tr	tr	+	0	0	0	0

Leucine 42								
Exp. Two	0	0	+	+	0	0	0	0
Exp. Three	0	0	0	0	0	0	0	0
Exp. Four	0	+	+	0	0	0	-	-
Exp. Five	+	+	0	0	-	-	-	-
Average	tr	tr	tr	tr	0	0	0	0

Isoleucine 4								
Exp. Two	0	0	++	+	0	0	0	++
Exp. Three	0	0	+++	++	0	0	+++	0
Exp. Four	0	0	0	0	0	0	-	-
Exp. Five	0	0	0	0	-	-	-	-
Average	0	0	+	+	0	0	+	+

Table 2. (Concl.)

:Charleston Gray: Black Diamond : Butternut :Cucumber 163213								
Experiments:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl								
Inositol 2								
Exp. Two	0	0	0	0	0	0	0	+++
Exp. Three	0	+	++	tr	0	0	0	0
Exp. Four	0	0	0	++	0	0	0	0
Exp. Five	tr	++	+++	+++	-	-	-	-
Average	0	+	+	++	0	0	0	+
Histidine 24								
Exp. Two	0	0	tr	+	0	0	0	0
Exp. Three	0	0	0	0	0	0	0	0
Exp. Four	0	0	0	0	0	0	-	-
Exp. Five	0	0	0	0	-	-	-	-
Average	0	0	0	tr	0	0	0	0
Serine 29								
Exp. Two	0	0	0	0	0	0	0	0
Exp. Three	0	++	0	0	0	0	0	0
Exp. Four	0	++	++	++	0	0	0	0
Exp. Five	+	++	0	0	-	-	-	-
Average	tr	++	tr	tr	0	0	0	0
Alanine 48								
Exp. Two	0	0	0	0	0	0	0	0
Exp. Three	0	++	0	0	0	0	0	0
Exp. Four	0	++	0	0	0	0	-	-
Exp. Five	0	0	0	0	-	-	-	-
Average	0	+	0	0	0	0	0	0
Valine 49								
Exp. Two	0	0	0	0	0	0	0	0
Exp. Three	0	0	0	++	0	0	0	0
Exp. Four	0	0	+	0	0	0	-	-
Exp. Five	0	0	0	0	-	-	-	-
Average	0	0	tr	tr	0	0	0	0

Seedlings of Butternut were immune to all mutants studied. In no case could virulence be restored. Cucumber 163213 appeared immune to all mutants, except Isoleucine 4. Results from this mutant in two experiments were somewhat different. Experiment two gave an immune rating for the non-supplemented seedlings, but the virulence was restored to ++ for the supplemented seedlings. Experiment three gave a rating of +++ for the non-supplemented seedlings and no infection of the supplemented ones. In both cases an average of + was the result. In one case out of three virulence of the Inositol 2 mutant was restored to +++.

Charleston Gray was immune to Pyrimidine 44, Pyrimidine 52, Proline 22, Isoleucine 4, Inositol 2, Histidine 24, Alanine 48 and Valine 49. Of these, the virulence of the Pyrimidine 52, Proline 22, Inositol 2 and Alanine 48 mutants was restored; the Pyrimidine 52 and Proline 22 mutants gave a very slight infection (tr) when supplemented, Inositol 2 and Alanine 48 gave a more distinct one (+). In one case out of four Leucine 42 and Serine 29 caused infection, and an increase in virulence was observed when supplemented with the appropriate amino acids.

Black Diamond was immune to Pyrimidine 44, Pyrimidine 52, Histidine 24 and Alanine 48. Of these, the virulence of the Pyrimidine 52 and Histidine 24 mutants was restored when supplemented. The mutants requiring proline, leucine, isoleucine and inositol produced distinct infection in two experiments out of four, and the two last mutants produced the most severe symptoms of all of them. The mutants requiring serine and valine infected Black Diamond seedlings only in one experiment each out of four. The degree of infection of these virulent mutants was the same when supplemented, except for Proline 22 and Inositol 2 which increased in virulence.

Table 3. Seedling infections obtained by races of *C. lagenarium* in experiments two, three, four, and five, Manhattan, Kansas, 1960.

Experiments : Charleston Gray : Black Diamond : Butternut : Cucumber 163213				
		Race I		
Exp. Three	0.00	4.00	0.00	0.00
Exp. Four	0.00	4.00	0.00	-
Exp. Five	0.00	1.50	-	-
Average	0.00	3.20	0.00	0.00
		Race II		
Exp. Three	4.00	5.00	0.00	0.00
Exp. Four	3.00	5.00	0.00	0.00
Exp. Five	3.00	5.00	-	-
Average	3.33	5.00	0.00	0.00
		Race III		
Exp. Three	contaminated	3.00	0.00	0.00
Exp. Four	0.00	3.00	0.00	-
Exp. Five	1.00	2.50	-	-
Average	0.50	2.83	0.00	0.00
		KS II		
Exp. Three	3.00	5.00	0.00	3.00
Exp. Four	2.00	5.00	0.00	0.00
Exp. Five	2.00	4.50	-	-
Average	2.33	4.83	0.00	1.50

In all experiments Charleston Gray, Butternut and cucumber 163213 appeared immune to Race I. Black Diamond showed moderate resistance. However, a variation occurred between experiments thus giving a susceptible rating in experiments three and four, and a resistant rating in experiment five. This difference may have been due to variations in humidity or temperature during incubation.

Butternut and cucumber 163213 were immune to Race II. Charleston Gray was classified as moderately resistant, ranging from moderate resistance in experiments four and five to susceptible in experiment three. Black Diamond was highly susceptible in all cases.

Butternut and cucumber 163213 were immune to Race III. Charleston Gray was slightly infected in experiment five and rated as highly resistant. The infection of Charleston Gray in experiment three was considered to be the result of contamination from an adjacent seedling inoculated with the virulent Race II. Black Diamond showed moderate resistance to Race III through all experiments.

Butternut was immune to the Kansas II strain again. Cucumber 163213 which was immune to the other races was rated as resistant in this case, as well as Charleston Gray. Black Diamond was highly susceptible.

Homogenates

Traces of growth of the Pyrimidine 44 mutant was observed both in the supplemented and non-supplemented epidermal tissue homogenates of Charleston Gray, while it was given a + rating in the same homogenates of Black Diamond. No growth response was obtained even in supplemented homogenates of Butternut and cucumber 163213.

No growth response of the Pyrimidine 52 mutant could be observed in non-supplemented or supplemented epidermal tissue homogenates of all four host varieties.

A positive growth response was obtained from the Proline 22 mutant in all tissue homogenates except the cucumber where only a trace of growth was observed. The same results were obtained for supplemented homogenates.

The epidermal tissue homogenates of Charleston Gray and 163213 apparently did not supply the Leucine 42 mutant with leucine required for growth. Mutant growth was obtained in the supplemented Charleston Gray homogenates, but not in the case of 163213. Homogenates of Black Diamond and Butternut did supply

Table 4. Mutant growth in epidermal tissue homogenates in experiments three and five, Manhattan, Kansas, 1960.

:Charleston Gray:Black Diamond : Butternut :Cucumber 163213								
Experiments:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl								
Pyrimidine 44								
Exp. Three	0	0	tr	tr	tr	tr	0	0
Exp. Five	+	+	+	+	0	0	-	-
Average	tr	tr	+	+	0	0	0	0
Pyrimidine 52								
Exp. Three	0	0	tr	tr	tr	tr	0	0
Exp. Five	0	0	0	0	0	0	-	-
Average	0	0	0	0	0	0	0	0
Proline 22								
Exp. Three	tr	tr	+	+	+	+	tr	tr
Exp. Five	+	++	+	++	contam.	-	-	-
Average	+	+	+	+	+	+	tr	tr
Leucine 42								
Exp. Three	0	tr	tr	tr	+	+	0	0
Exp. Five	tr	+	tr	+	tr	tr	-	-
Average	0	+	tr	+	+	+	0	0
Isoleucine 4								
Exp. Three	0	tr	0	+	0	tr	0	tr
Exp. Five	0	++	0	++	0	++	-	-
Average	0	+	0	+	0	+	0	tr
Inositol 2								
Exp. Three	+	+	tr	++	+	++	+	++
Exp. Five	tr	++	tr	++	0	++	-	-
Average	+	+	tr	++	tr	++	+	++
Histidine 24								
Exp. Three	+	++	+	+	+	+	+	++
Exp. Five	+	++	+	++	+	+	-	-
Average	+	++	+	+	+	+	+	++
Serine 29								
Exp. Three	+	++	+	++	+	++	+	++
Exp. Five	+	++	+	++	+	++	-	-
Average	+	++	+	++	+	++	+	++

Table 4. (Concl.)

:Charleston Gray:Black Diamond : Butternut :Cucumber 163213								
Experiments:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl								
Alanine 48								
Exp. Three	+	++	tr	+	+	+	+	+
Exp. Five	tr	++	tr	++	0	+	-	-
Average	+	++	tr	+	tr	+	+	+
Valine 49								
Exp. Three	0	tr	tr	tr	0	tr	+	tr
Exp. Five	0	0	0	0	0	0	-	-
Average	0	0	0	0	0	0	+	tr

leucine for mutant growth.

There was no growth response of the Isoleucine 4 mutant in epidermal tissue homogenates of all four host varieties. In all cases, however, positive responses were obtained from the isoleucine supplement.

A positive growth response was obtained by the Inositol 2 mutant both in supplemented and non-supplemented epidermal tissue homogenates of all four varieties. The differences in response between the host varieties could not be considered significant. Similar results were obtained with the Histidine 24, Serine 29 and Alanine 48 mutants.

The amino acid valine was supplied only by the epidermal tissue homogenate of cucumber 163213. Less growth (tr) was obtained in supplemented test tubes.

A positive growth response was observed with Race I in the epidermal tissue homogenate of cucumber 163213. In this growth medium all other races showed no response. Traces of growth of Race I were observed in homogenates of Charleston Gray and Butternut. However, no response could be observed in homogenate of Black Diamond.

Table 5. Growth of races in epidermal tissue homogenates in experiments three and five, Manhattan, Kansas, 1960.

<u>Experiments : Charleston Gray : Black Diamond : Butternut : Cucumber 163213</u>				
Race I				
Exp. Three	contam.	0	tr	+
Exp. Five	tr	tr	tr	-
Average	tr	0	tr	+
Race II				
Exp. Three	tr	tr	tr	0
Exp. Five	tr	tr	tr	-
Average	tr	tr	tr	0
Race III				
Exp. Three	0	0	tr	0
Exp. Five	tr	tr	tr	-
Average	0	0	tr	0
KS II				
Exp. Three	0	tr	tr	0
Exp. Five	tr	tr	tr	-
Average	0	tr	tr	0

Traces of growth of Race II were observed in homogenates of Charleston Gray, Black Diamond, and Butternut.

Inconsistent results were obtained with Race III in epidermal tissue homogenates of Charleston Gray and Black Diamond. A trace of growth was noted in the Butternut homogenate.

No growth of the Kansas II strain was obtained in homogenates of Charleston Gray and 163213 but traces of growth were obtained in those of Black Diamond and Butternut.

The mutant Pyrimidine 44 (Table 6) responded positively (+) only to the epidermal tissue homogenate of Black Diamond. No growth was observed in homogenates of the other host varieties. Growth could not be obtained by supplementing pyrimidine.

Growth of the Pyrimidine 52 mutant was observed in sub-epidermal tissue homogenates of Charleston Gray, Black Diamond and Butternut and a trace in that of cucumber 163213. The same mutant gave a trace response in the whole tissue homogenates of Black Diamond and Butternut, none in the Charleston Gray and cucumber 163213.

The Proline 22 mutant responded with distinct growth (++) both in non-supplemented and supplemented homogenates of Charleston Gray, Black Diamond and Butternut and a trace of growth was observed in the cucumber homogenate.

The Leucine 42, Isoleucine 4, Inositol 2, Histidine 24, and Serine 29 mutants responded with up to (+++) growth ratings in non-supplemented and supplemented homogenates of all four host varieties.

Differences in response between host varieties were obtained with the alanine and valine requiring mutants. Alanine 48 responded with distinct growth (++) consistently through both experiments in Black Diamond homogenates. The same mutant responded with complete growth (+++) in the whole tissue and with none in the sub-epidermal homogenates of Charleston Gray. Variations of growth from + to ++ were obtained with the Alanine 48 mutant in the homogenates of Butternut and cucumber 163213.

The Valine 49 mutant responded consistently with complete growth in homogenates of Butternut. In whole tissue homogenates of Charleston Gray and cucumber 163213 the same mutant responded with growth ratings of +++ and with only + in the sub-epidermal tissue homogenates of the same host varieties, giving an average ++. The Valine 49 mutant produced a + in the homogenates of Black Diamond.

Table 6. Mutant growth in whole and sub-epidermal tissue homogenates in experiments two and three, Manhattan, Kansas, 1960.

:Charleston Gray:Black Diamond : Butternut :Cucumber 163213								
Experiments:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl								
Pyrimidine 44								
Exp. Two	0	0	0	0	0	0	0	tr
Exp. Three	0	0	+	tr	contam.		tr	0
Average	0	0	tr	0	0	0	0	0
Pyrimidine 52								
Exp. Two	0	0	tr	0	tr	0	0	tr
Exp. Three	+	+	+	+	+	+	tr	tr
Average	tr	tr	+	tr	+	tr	0	tr
Proline 22								
Exp. Two	++	++	++	++	++	++	+	++
Exp. Three	++	++	++	++	++	++	0	tr
Average	++	++	++	++	++	++	tr	+
Leucine 42								
Exp. Two	++	++	++	++	++	++	++	++
Exp. Three	++	contam.	++	++	++	++	+	++
Average	++	++	++	++	++	++	+	++
Isoleucine 4								
Exp. Two	++	+++	++	++	++	++	++	++
Exp. Three	++	++	+	++	++	++	++	++
Average	++	++	+	++	++	++	++	++
Inositol 2								
Exp. Two	+++	+++	+++	+++	+++	+++	++	++
Exp. Three	+	contam.	++	+++	+++	+++	++	++
Average	++	+++	++	+++	+++	+++	++	++
Histidine 24								
Exp. Two	+++	+++	+++	++	++	++	++	+++
Exp. Three	tr	++	++	++	+++	+++	++	++
Average	++	++	++	++	++	++	++	++
Serine 29								
Exp. Two	+++	++	++	++	++	++	++	++
Exp. Three	++	++	++	+++	+++	+++	+	+
Average	++	++	++	++	++	++	+	+

Table 6. (Concl.)

:Charleston Gray:Black Diamond : Butternut :Cucumber 163213								
Experiments:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl:non-suppl:suppl								
Alanine 48								
Exp. Two	+++	++	++	++	+	+	+	++
Exp. Three	0	+	++	++	++	++	++	++
Average	+	+	++	++	+	+	+	++
Valine 49								
Exp. Two	+++	+++	+	++	+++	+++	+++	++
Exp. Three	+	tr	+	+	+++	+++	+	++
Average	++	++	+	+	+++	+++	++	++

Table 7. Growth of races in whole and sub-epidermal tissue homogenates in experiments two and three, Manhattan, Kansas, 1960.

Experiments : Charleston Gray : Black Diamond : Butternut : Cucumber 163213				
Race I				
Exp. Two	+	++	++	++
Exp. Three	+++	++	++	++
Average	++	++	++	++
Race II				
Exp. Two	++	++	++	++
Exp. Three	tr	++	+++	++
Average	+	++	++	++
Race III				
Exp. Two	++	+++	+++	++
Exp. Three	++	+	+++	tr
Average	++	++	+++	+
KS II				
Exp. Two	++	++	++	+
Exp. Three	+	++	+++	+
Average	+	++	++	+

Race I produced a growth rating of ++ in homogenates of all host varieties.

Similar ratings were obtained with Race II in the homogenates of Black Diamond, Butternut and cucumber 163213. However, it responded with less growth (+) in the homogenates of Charleston Gray.

Race III responded with ++ growth ratings in homogenates of both watermelon varieties, with consistent +++ for Butternut and only + for 163213.

The Kansas II strain produced growth in homogenates of Charleston Gray and 163213, but more so in homogenates of Black Diamond and Butternut.

DISCUSSION

Growth responses of mutants and races of Colletotrichum lagenarium cultured 1) on the surface of intact seedlings, 2) in epidermal and 3) whole or sub-epidermal tissue homogenates were observed in this study.

An important observation was that the Leucine h2 and Serine 29 mutants were virulent to seedlings of susceptible and resistant watermelon varieties (Table 2). However, somewhat less growth of the Leucine h2 mutant was obtained in the epidermal tissue homogenate of Charleston Gray, while there was no difference in the growth of the Serine 29 mutant (Table 4). The Leucine h2 and Serine 29 mutants grew in whole and sub-epidermal tissue homogenates of both varieties very similarly (Table 6). Charleston Gray was moderately resistant to susceptible to Race II and resistant to moderately resistant to KS II. These differences might possibly be related to the presence of available leucine and serine in the host tissues.

The highest degree of mutant infection was obtained with the Isoleucine 4 and Inositol 2 mutants which were virulent to Black Diamond seedlings. In one

experiment a trace of growth of the Inositol 2 mutant was observed on non-supplemented seedlings of Charleston Gray compared with a ++ rating on supplemented seedlings. Obviously, the susceptibility in Black Diamond to these mutants is related to available amounts of isoleucine and inositol, while the resistance in Charleston Gray might possibly be due to a lack of or non-availability of these nutrients. Growth of these two mutants in whole and sub-epidermal tissue homogenates of all varieties was quite similar (++ to +++). In epidermal tissue homogenates of both varieties growth of the Isoleucine 4 and Inositol 2 mutants was less (0 to +). A significant difference in rating was obtained between the Isoleucine 4 and Inositol 2 mutants with the latter giving the higher reading. It is evident that whole and sub-epidermal tissue homogenates in all cases except for the Pyrimidine 44 mutant supplied the required nutrients (Table 6). Comparatively less mutant growth was obtained in the epidermal tissue homogenates containing the Isoleucine 4 mutant which produced no growth and Inositol 2 a trace to +. It seems that the growth of these two mutants in the homogenates of resistant and susceptible hosts is similar. It is suggested that the difference in resistance of the intact seedlings to the mutants is due to an inhibition mechanism effective only in resistant growing plants, while a nutritional resistance is present to some degree in susceptible plants also.

The fact that the Alanine 48 mutant appeared avirulent to seedlings of all four host varieties but responded positively to both types of homogenates supports the assumption that resistance must be sought in the growing plant, not in macerated live tissues. Interesting results were obtained with the Valine 49 mutant. Butternut seedlings were immune to this, and the other mutants as well. Valine 49 did not grow in the epidermal tissue homogenate

of the same host, only in one experiment a trace of growth could be seen, while in whole and sub-epidermal tissue homogenates complete growth was obtained consistently. The same trend was observed for the Valine 49 mutant in the homogenates of all other host varieties except 163213. Thus, valine, evidently, was not available in the epidermal layers of any of the remaining varieties. In general less mutant growth was obtained in epidermal than in whole or sub-epidermal tissue homogenates although not so pronounced as in the case of Butternut in respect to the Valine 49 mutant. Less growth was obtained with Valine 49 (Table 4, 163213) and Pyrimidine 44 and 52 (Table 6, Black Diamond and Butternut) in supplemented homogenates than in non-supplemented which is unexplainable.

SUMMARY AND CONCLUSION

The nature of chemical resistance to anthracnose in cucurbits was investigated in five experiments conducted between February and July 1960. The work was carried out in the Horticultural Greenhouse and in the Laboratories of the Department of Botany and Plant Pathology. Infection and growth responses of mutants and races of Colletotrichum lagenarium when cultured 1) on the surface of intact seedlings, 2) in epidermal tissue homogenates and 3) in whole or sub-epidermal tissue homogenates were observed. Differences in reactions of the ten mutants and four races were found to Charleston Gray, Black Diamond, Butternut, and cucumber 163213.

Butternut seedlings were immune to all mutants and all races of C. lagenarium. In no case could virulence be restored. A trace to a + growth was observed in epidermal tissue homogenates of all mutants except Pyrimidines 44 and 52, Isoleucine 4 and Valine 49, which gave no growth response. All

racess responded with a trace in the same type of homogenates. For whole and sub-epidermal tissue homogenates a range from + to +++ was observed for the various mutants and races. The Pyrimidine 44 mutant alone did not show any response. A great difference was noted in mutant and race growth in epidermal and whole or sub-epidermal tissue homogenates of Butternut.

Cucumber 163213 seedlings were immune to all mutants except Isoleucine 4, and to all races except KS II. Inositol 2 was the only non-pathogenic mutant where virulence could be restored. From a trace to a + growth rating was obtained in non-supplemented epidermal tissue homogenates from all mutants except Pyrimidines 44 and 52, Leucine 42 and Isoleucine 4. However, the virulence of Isoleucine 4 was restored in supplemented homogenates. Only Race I grew in a similar type of homogenate. For whole or sub-epidermal tissue homogenates, growth ranges from trace to ++ were observed for the mutants, and from + to ++ for races. The Pyrimidine 44 and 52 mutants did not show any response but when supplemented the latter produced a trace of growth.

Seedlings of Charleston Gray appeared immune to all mutants except Leucine 42 and Serine 29. It might be suggested that the presence of the amino acids leucine and serine in the host tissues plays a role in the ability of Race II and KS II to infect Charleston Gray. The virulences of the non-pathogenic mutants Pyrimidine 52, Proline 22, Inositol 2 and Alanine 48 were restored. Charleston Gray was immune only to Race I. The response of Charleston Gray to Race II ranged from moderately resistant to susceptible. High resistance was recorded to Race III, and a range from resistant to moderately resistant to KS II. A trace to + response was observed in epidermal tissue homogenate with all mutants except Pyrimidine 52, Leucine 42, Isoleucine 4

and Valine 49. When supplemented growth was obtained with Leucine 42 and Isoleucine 4. A trace of growth was observed with the races in the same type of homogenates except for Race III and KS II which in only one experiment rated a trace, resulting in a zero growth average. For whole and sub-epidermal tissue homogenates a growth range from + to ++ was observed for all mutants and races except for Pyrimidine 44 where no response was obtained either in supplemented or non-supplemented test tubes. The Pyrimidine 52 mutant showed a trace of growth in both instances.

Seedlings of Black Diamond were infected by all mutants except Pyrimidine 44 and 52, Histidine 24 and Alanine 48. When supplemented infection was obtained with Pyrimidine 52 and Histidine 24. The rating of races ranged from moderately resistant (Race III) to highly susceptible (Race II). Trace to + growth ratings were obtained in epidermal tissue homogenate for all mutants except Pyrimidine 52, Valine 49 and Isoleucine 4 for which growth alone was restored. Traces of growth were obtained for Races I and III in only one experiment. For whole and sub-epidermal tissue homogenate, a range from + to ++ was obtained for most of the mutants, only the Pyrimidine 44 showed a trace. Races responded with a ++ in the same type of homogenate.

From this series of experiments, the following conclusions might be drawn. The difference in resistance between varieties to biochemical mutants and to races of Colletotrichum lagenarium can clearly be seen on inoculated intact seedlings. The results obtained from experiments with epidermal tissue homogenates can be related to results obtained by wounding (35). Since in most cases, consistently less growth was obtained in epidermal than in whole or sub-epidermal tissue homogenates some kind of barrier, probably a lack, or too low concentration of the required nutrilité may be assumed to occur in

the epidermal tissue homogenates of both resistant and susceptible plants.

Results of this study support the "nutrition-inhibition hypothesis of pathogenecity". Since virulences of the mutants Pyrimidine 52 non-pathogenic to Charleston Gray and Black Diamond, Proline 22 non-pathogenic to Charleston Gray, Inositol 2 non-pathogenic to Charleston Gray and 163213, Histidine 24 non-pathogenic to Black Diamond, and Alanine 48 non-pathogenic to Charleston Gray and 163213, Histidine 24 non-pathogenic to Black Diamond, and Alanine 48 non-pathogenic to Charleston Gray could be restored by exogenous supply to intact seedlings it has been shown that nutrition of the fungus plays at least a role in the resistance. Another important phase of resistance of cucurbits to C. lagenarium should be sought in living seedlings where certain inhibitors not detectable in macerated tissue may be found.

ACKNOWLEDGMENTS

The author owes sincere gratitude to her major professor Dr. Charles V. Hall, Department of Horticulture, for his valuable assistance, guidance in writing the manuscript, for providing greenhouse facilities, and for taking the photographs. The author is also grateful to Dr. C. L. Kramer, Department of Botany and Plant Pathology, for providing laboratory facilities, for help and cooperation. Deep indebtedness is expressed to Dr. William F. Pickett, Head of the Department of Horticulture, for interest and encouragement. The author is also thankful to the Institute of International Education which assistance made it possible for the author to materialize these studies.

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CHEMICAL NATURE OF ANTHRACNOSE RESISTANCE IN CUCURBITS

by

ANNA CECILIA MATHILDA BREDENBERG

B. Sc. (Ag.), University of Helsinki, Finland, 1957

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1961

This study was designed to reveal new evidence concerning the chemical nature of anthracnose resistance in cucurbits. Five experiments were completed between February and July 1960 in the Horticultural Greenhouse and in the Laboratories of the Department of Botany and Plant Pathology.

Seedlings of Charleston Gray, Black Diamond, Butternut, and cucumber 163213 were grown in the greenhouse for all experiments.

In the greenhouse experiments seedlings were inoculated with ten mutants and four races of C. lagenarium, 1) by applying inoculum to cheese cloth squares placed on the leaves, and 2) by means of the "dunking" technic. In both cases nutrilites required by the respective mutants requiring pyrimidines, the amino acids proline, leucine, isoleucine, histidine, serine, alanine and valine, and the vitamin inositol were applied to cheese cloth squares on the leaves. Seedlings were incubated in a moist chamber for 14-21 days before ratings were made according to "0 to +++" and "0 to 5" scales.

In the laboratory three types of homogenates were prepared by grinding in a Waring blender: 1) whole tissues of seedlings, including leaves stems and roots, 2) the epidermal layer of stems and petioles of seedlings, and 3) sub-epidermal tissues from peeled stems and petioles. The homogenates were transferred to sterile test tubes using a Multifit syringe. After inoculation 0.5 cc. of the required nutrilites were added to the test tubes to be supplemented. All test tubes were incubated in the laboratory at room temperature. Ratings were made after 5 to 8 days according to a "0 to +++" scale.

The Pyrimidine 44 and 52, Histidine 24 and Alanine 48 mutants were avirulent to seedlings of all host varieties. When supplemented, Pyrimidine 52 was virulent to seedlings of Charleston Gray and Black Diamond, Histidine 24 to Black Diamond, and Alanine 48 to Charleston Gray. Proline 22 was only

virulent to Black Diamond, but its virulence could be restored on seedlings of Charleston Gray. Leucine 42 was virulent to both Black Diamond and Charleston Gray; Isoleucine 4 to Black Diamond and 163213; Inositol 2 to Black Diamond, but its virulence was restored on supplemented seedlings of Charleston Gray and 163213. Serine 29 was virulent to both Black Diamond and Charleston Gray, whereas Valine 49 was only virulent to Black Diamond.

Race I was avirulent to all seedlings except Black Diamond. Race II was avirulent to Butternut and 163213 but highly virulent to Black Diamond and moderately to Charleston Gray. Race III was avirulent to Butternut and 163213, virulent to Black Diamond, and slightly virulent to Charleston Gray seedlings. KS II showed a variable degree of virulence to all host varieties, except to Butternut.

In many cases mutant and race growth was similar in homogenates of resistant and susceptible host tissue with the exceptions of the Pyrimidine 44, Leucine 42, Alanine 48 and Valine 49 mutants. The Pyrimidine 44 mutant produced only a trace of growth in epidermal tissue homogenate of Charleston Gray, whereas for Black Diamond a + rating was obtained. No response was obtained from Butternut and 163213. Leucine 42 responded with a trace of growth to Black Diamond, and a + to Butternut, while no response was obtained in the epidermal tissue homogenates of Charleston Gray and 163213, although a growth response to the former was obtained when supplemented. Alanine 48 produced more mycelial growth in homogenates of Charleston Gray and 163213 than in Black Diamond and Butternut. The growth response of Valine 49 was zero, and similar in all varieties except 163213 where a positive response was observed. There were no significant differences in the response of races between varieties, except for 163213 with a + to Race I and 0 to the other races.

Differences in mutant growth in whole and sub-epidermal homogenate were represented by a positive rating for Pyrimidine 44 in Black Diamond and a negative response for the remaining varieties. Pyrimidine 52 showed a trace of growth in homogenate of Charleston Gray, a + in Black Diamond and Butternut, and no growth in 163213. Proline 22 made distinct growth in homogenates of all varieties except for a trace in 163213. Alanine 48 was rated as a + in Charleston Gray, Butternut and 163213, and ++ in Black Diamond homogenates. Valine 49 was rated a + in homogenates of Black Diamond, ++ in Charleston Gray and 163213 and +++ in Butternut. In general the weaker response was obtained in 163213 when compared to whole or sub-epidermal tissue homogenates of the three other varieties.

Much less growth was obtained in epidermal than in whole or sub-epidermal tissue homogenates of all varieties with the exception of the Pyrimidine 44 mutant which grew more in the former than in the latter.